Jl S. Afr. Bot. 46 (2): 147–155 (1980)

FLAVONOID GLYCOSIDES OF *LEUCADENDRON* AND THEIR CHEMOTAXONOMIC SIGNIFICANCE

C. W. Glennie*

(Department of Chemistry, University of the Witwatersrand, Johannesburg)

Abstract

The flavonoid content of leaf material of 59 taxa of the genus *Leucadendron* (Proteaceae) was examined by two-dimensional paper chromatography. Most samples contained the 3-0-galactosides and 3-0-glucosides of quercetin and isorhamnetin while the rutinosides of these two aglycones were more restricted in their distribution, being found mainly in the *Leucadendron* section of this species. The 3-0-galactoside and 3-0-glucoside of myricetin were very restricted occurring only in a few species of the other section, *Alatosperma*. Cyanogenic compounds were detected for the first time in the Proteoideae (the South African sub-family of Proteaceae) and arbutin was also present in a limited number of species. The phenolic dilactone, leucodrin, was found in all taxa surveyed. The taxonomic significance of these distribution patterns as well as the phylogenetic implications are discussed.

Uittreksel

FLAVANOIEDE GLIKOSIEDES VAN *LEUCADENDRON* EN HULLE CHEMOTAKSONOMIESE BELANGRIKHEID

Die flavanoiede inhoud van die blaar van 59 taksons van die genus *Leucadendron* (Proteaceae) was ondersoek deur middel van twee-dimensionele papier chromatografie. Meeste van die monsters bevat die 3-0-galaktosiede en 3-0-glukosiede van kwersetien en isorhamnetien terwyl die rutinosiede van hierdie twee aglikone baie meer beperk was in hulle verspreiding aangesien hulle hoofsaaklik gevind word in die *Leucadendron* afdeling van spesies. Die 3-0-galaktosied en 3-0-glukosied van myrisetien was baie beperk en kom net voor in 'n paar spesies van die ander afdeling *Alatosperma*. Sianogeniese stowwe was vir die eerste keer gevind in die Proteoideae (die Suid-Afrikaanse onderafdeling van die Proteaceae) en arbutien was ook teenwoordig in beperkte getalle spesies. Die fenoliese dilaktoon, leucodrin, is ook gevind in al die taksons wat ondersoek was. Die taksonomiese belangrikheid en ook die filogenetiese gevolgtrekkings word bespreek.

Introduction

The genus *Leucadendron* was first defined as such in 1809 when Salisbury divided *Protea* into different genera. *Leucadendron* as well as *Protea* belong to the Proteoideae sub-family of the Proteaceae; it is in this sub-family where most of the South African members of the Proteaceae are found. Robert Brown in 1810 divided *Leucadendron* into several sections and with modification this division has held until the present time. The most recent taxonomic treatment of the genus *Leucadendron* was done by Ion J. M. Williams (1972) in which he recognized 91

---

*Present address: Sorghum Beer Unit, National Food Research Institute, Council for Scientific and Industrial Research, Pretoria.

Accepted for publication 11th June, 1979.

taxa. Williams divided the genus into two sections and 14 sub-sections. Included in his monograph is a worthwhile and interesting section on the taxonomic history of *Leucadendron*.

During a recent survey of some South African members of the Proteaceae for flavonoids and other phenolic compounds, several species of *Leucadendron* were examined. This paper reports the results of this examination and discusses the findings from a chemotaxonomic point of view. Several other workers have looked at the various phenolic compounds found in *Leucadendron* spp. at different times and this paper attempts to incorporate their results in the chemotaxonomic discussion.

Van Oudtshoorn (1963) found leucoanthocyanins in all 11 species of *Leucadendron* he examined, while Elsworth and Martin (1971) also found these compounds present in the 27 species of their study. The combined total of these two studies is 38 different *Leucadendron* spp. which produce anthocyanidins on acid hydrolysis. Quercetin and kaempferol were both found to have widespread distribution in these two studies while myricetin did not. Elsworth and Martin found myricetin present in only 5 of the 27 species examined, while Van Oudtshoorn reported 6 out of 11 species contained myricetin. Van Oudtshoorn also reported the presence of arbutin in all samples of unhydrolysed leaf material and hydroquinone present in the diethyl ether extracts of hydrolysed material.

There has been no report of cyanogenic compounds being found in *Leucadendron* spp.; in fact, the only report of HCN being produced by any plant in this sub-family was *Protea cynaroides* (Hegnauer, 1959). Another compound with restricted distribution is the phenolic dilactone leucodrin. Its structure was firmly established by Perold and Pachler (1966) and it has now been found in every species of *Leucadendron* examined and is characteristic of this genus (Plouvier, 1964; Murray and Bradshaw, 1966). Leucodrin has also been found in a few species of *Leucospermum* but the compound which is characteristic of this genus is conocarpin which is a diastereoisomer of leucodrin (Perold, Hodgkinson, Howard and Kruger, 1972).

The reasons that flavonoids are so widely used as taxonomic guides have been well documented by Harborne (1967, 1975). In the previous studies already mentioned, where flavonoid markers were used in chemotaxonomic studies involving this genus, only the aglycones were isolated and identified. In an attempt to broaden the scope of this type of study, the present work used unhydrolysed aqueous methanolic extracts of leaf material which allowed the flavonoid glycosides to be isolated and identified. This study is the first to give the genus *Leucadendron* an in-depth treatment from the chemotaxonomic point of view. Besides the flavonoid glycosides, the presence of arbutin and cyanogenic compounds, as well as leucodrin, are reported.

METHODS

The plant material was collected from the Botanical Research Institute, Pretoria and the National Botanic Gardens, Kirstenbosch. Voucher specimens are deposited in the herbaria of these two institutions. The material was either air-dried and milled before extraction with 80% methanol or the fresh material was ground in a Waring Blendor directly with 80% methanol. After extraction, the flavonoids were separated by paper chromatography and identified by the standard procedures of R_f values, acid hydrolysis and UV spectroscopy (Harborne, 1967). R_f values and UV spectral data for newly isolated flavonoids are found in Table 1; for information regarding other flavonoids reported in Table 2, but not described in Table 1 see Glennie (1979). All aglycone structures were confirmed by mass spectroscopy.

To determine the patterns of flavonoids present in the different plant samples, each sample was chromatographed two-dimensionally on Whatman No. 1 paper using PhOH (phenol : water–500:125) and 15% HOAc. The dried chromatograms were dipped in a solution of 3% $AlCl_3$ in methanol before air drying and viewing under a UV light. This converted the flavonoids from dull absorbing spots into bright yellow spots which were more easily and reliably scored.

All unhydrolysed plant extracts were examined for the presence of leucodrin, arbutin and hydroquinone by paper chromatography. Whatman No. 1 paper was pretreated with 10% glycerol in methanol and after a known amount of plant extract was spotted it was developed in BuOH : Tol (butanol : toluene–1:1 and

TABLE 1

R_f and spectral characteristics of some flavonoid glycosides of *Leucadendron*

	R_f (x 100 on Whatman No. 1) in			
Glycoside	BAW	15% HOAc	H_2O	Phenol*
Quercetin-3-0-rutinoside	40	53	28	46
Myricetin-3-0-galactoside	12	20	11	19
Myricetin-3-0-glucoside	14	26	08	29

	λ_{max} (nm) in 80% MeOH	
Glycoside	Band II	Band I
Quercetin-3-0-glycoside	256(265)†	358
Myricetin-3-0-glycoside	264	356

	$\Delta\lambda_{max}$ (nm) in			
Glycoside	NaOAc Band II	$AlCl_3$ Band I	NaOMe Band I	H_3BO_4 Band I
Quercetin-3-0-glycoside	18	62	42	22
Myricetin-3-0-glycoside	6	67	decomposes	20

*BAW-organic layer of butanol : acetic acid : water (4:1:5);
15% HOAc—15% acetic acid; Phenol—500 g phenol in 125 g water.
†inflection.

TABLE 2
Distribution of phenol glycosides in *Leucadendron* species

Species						Compounds					
Section LEUCADENDRON	QGal	QGlu	QRut	IGal	IGlu	IRut	IAra	MGal	MGlu	CNg	Arb
Sub-section VILLOSA Williams											
L. coriaceum Phillips & Hutch.	+	+		+	+						
L. brunioides Meisn.	+	+		+	+						
L. stellare (Sims) Sweet	+			+						+	
L. levisanus (L) Berg.	+	+		+	+						
L. linifolium (Jacq.) R. Br.	+	+		+	+						
L. galpinii Phillips & Hutch.	+			+		+					
L. dubium Buek ex Phillips & Hutch.	+	+		+	+						
Sub-section MEMBRANACEA Williams											
L. remotum Williams	+	+		+	+						
L. pubescens R. Br.			+			+					
L. arcuatum (Lam.) Williams		+	+	+		+					
Sub-section NERVOSA Williams											
L. nervosum Phillips & Hutch.	+	+		+	+						
Sub-section LEUCADENDRON											
L. dregei E. Mey ex Meisn.			+								+
L. album (Thunb.) Fourcade	+	+		+	+						
L. rubrum Burm f. Prodr.	+	+		+	+						
L. argenteum (L.) R. Br.	+										
Sub-section CUNEATA Williams											
L. verticillatum (Thunb.) Meisn.	+			+	+						
L. corymbosum Berg.				+	+						
L. laxum Williams			+	+	+	+					
Sub-section NUCIFERA Williams											
L. sheilae Williams	+	+		+	+					+	
L. glaberrimum (Schltr.) Compton	+	+		+	+					+	
L. loranthifolium (Salisb. ex Knight) Williams	+	+		+	+					+	
L. roodii Phillips	+	+		+	+					+	
L. cadens Williams			+							+	
L. gydoense Williams			+			+				+	
L. sessile R. Br.			+							+	
L. daphnoides (Thunb.) Meisn.	+	+		+	+						+
L. barkerae Williams			+							+	
L. burchellii Williams			+							+	
L. tradouwense Williams			+			+				+	
L. tinctum Williams			+			+				+	

Sub-section VENTRICOSA Williams											
L. chamelaea (Lam.) Williams	+		+			+					
Section ALATOSPERMA Williams											
Sub-section TRIGONA Williams											
L. floridum R. Br.	+	+		+	+						
L. uliginosum R. Br.	+	+	+		+						
L. uliginosum ssp. *glabratum* Williams	+	+	+		+						
L. rourkei Williams	+	+		+	+						
L. conicum (Lam.) Williams	+	+		+	+						
L. salicifolium (Salisb.) Williams	+	+		+	+		+			+	
Sub-section BRUNNEOBRACTEATA Williams											
L. microcephalum (Gandoger) Gandoger & Schinz	+	+		+	+			+	+		+
Sub-section ALATA Williams											
L. xanthoconus (O. Ktze.) K. Schum.	+	+		+	+			+	+		
L. lanigerum Buek ex Meisn.											
L. var *laevigatum* Meisn.		+									+
L. modestum Williams		+				+					+
L. diemontianum Williams	+	+		+	+		+				+
L. flexosum Williams	+	+		+	+						+
L. salignum Berg.	+	+		+	+					+	+
L. foedum Williams	+	+		+	+						
L. procerum (Salisb. ex Knight) Williams	+	+		+	+						
L. discolor Phillips and Hutchinson	+	+		+	+						+
L. cryptocephalum Guthrie	+			+						+	+
L. eucalyptifolium Buek. ex Meisn.	+			+							+
L. gandogeri Schinz ex Gandoger	+	+		+	+			+	+	+	
L. strobilinum (L.) Druce	+	+		+							
L. spissifolium (Salisb. ex Knight) Williams	+	+	+	+	+	+				+	
L. spissifolium ssp. *fragrans* Williams	+	+		+	+	+				+	
L. spissifolium ssp. *phillipsii* (Hutchinson) Williams	+	+		+	+					+	
L. spissifolium ssp. *natalense* (Thode & Gilg.) Williams	+	+		+	+					+	
L. spissifolium ssp. *oribinum* Williams				+	+						
Sub-section COMPRESSA Williams											
L. teretifolium (Andrews) Williams	+	+		+	+	+				+	
L. nobile Williams	+	+		+	+					+	
L. muirii Phillips	+	+								+	
L. platyspermum R. Br.	+			+		+					+

Key: QGal Quercetin 3-galactoside; QGlu quercetin 3-glucoside;
QRut quercetin 3-rutinoside; IGal isorhamnetin 3-galactoside;
IGlu isorhamnetin 3-glucoside; IRut isorhamnetin 3-rutinoside;
IAra isorhamnetin 3-arabinoside; MGal myricetin 3-galactoside;
MGlu myricetin 3-glucoside; CNg cyanogenic compound;
Arb arbutin.

saturated with H_2O). After air-drying, the chromatograms were sprayed with Pauly's Reagent (0,5 % solution of diazotised sulphanilic acid in 10 % aqueous Na_2CO_3). The extracts were also examined for cyanogenic compounds using methods previously described (Glennie and Davidson, 1978). The presence or absence of hydroquinone is not reported in this paper as it is probably an artifact produced by the breakdown of arbutin.

DISCUSSION

The distribution of the different phenolic glycosides throughout 12 of the 14 sub-sections is shown in Table 2. The table covers 59 of the 91 taxa suggested by Williams (1972) and the table is set out according to his arrangement of the genus. All the plants listed have been screened for all the compounds listed and only positive results presented. Blanks in the table represent negative results.

The most common flavonoid aglycones found in this study were quercetin and isorhamnetin and they were found singly or together in every sample examined. The glucosides and galactosides of these aglycones were the most prominent glycoside forms and occurred in over 50 % of the taxa listed in Table 2. Another reasonably common glycoside was the rutinoside of both quercetin and isorhamnetin. The other flavonoids listed occurred rarely.

The results from this chemotaxonomic study support the division of the genus *Leucadendron* into sections and sub-sections as suggested by Williams. The major flavonoids found in most sub-sections were the galactosides and glucosides of quercetin and isorhamnetin. The rutinosides of these two aglycones were found mainly in the *Leucadendron* section while the myricetin glycosides were found only in the *Alatosperma* section. This latter point is contrary to Williams' work as he suggested that this section contains the more advanced species while myricetin has traditionally been treated as a primitive character.

Sub-section *Nucifera* is characterized by containing mainly the rutinosides of quercetin and isorhamnetin with fewer species containing the flavonol galactosides and glucosides. Another chemical marker delimiting this sub-species is cyanogenesis which is widespread throughout. Cyanogenesis also characterizes the sub-sections *Alata* and *Compressa* where it is widespread. Species of *Alata* are also characterized by containing arbutin while most other sub-sections do not.

L. spissifolium is found in the *Alata* sub-section and this species is broken down into several sub-species most of which are reported in Table 2. The table shows that most of the sub-species contain only the galactosides and glucosides of the two flavonol aglycones while two sub-species contain the rutinosides as well. This chemical evidence supports Williams' grouping of the sub-species. The ssp. *oribinum* contains only isorhamnetin and also differs from the other sub-species in that it does not contain cyanogenic compounds.

Placed next to the *Alata* sub-section is the *Compressa* sub-section. This sub-section has the usual complement of flavonoids but like the species in *Alata* some species contain HCN producing compounds and one species even contains arbutin.

It is of interest that this is the first genus of the sub-family Proteoideae (contains predominantly South African species of Proteaceae) which shows a positive reaction when tested for cyanogenic compounds. Proteas growing in the summer rainfall region (Glennie and Davidson, 1978) and all species of *Leucospermum* examined (Glennie, 1979) gave negative results when tested for cyanogenesis. The ability to produce HCN appears to predominate in the Grevilleoideae which is the sub-family which contains mostly Australian species. Hegnauer (1964) reported 16 different species from Grevilleoideae which gave positive results when tested for HCN production.

In the genus *Leucadendron* cyanogenic compounds are restricted to five of the ten sub-sections tested. In the sub-section *Nucifera* all species examined were positive for HCN except *L. daphnoides* which again was the only species examined from this sub-section to contain arbutin. Sub-sections *Alata* and *Compressa* are the only other sub-sections where cyanogenesis is common.

Arbutin occurs sporadically throughout many sub-sections of *Leucadendron* occurring mainly in the sub-section *Alata*; it occurs in 8 out of 14 species examined and tends not to be present in those species which are HCN producers. Van Oudtshoorn reported arbutin present in all 11 species of *Leucadendron* he examined but he also reported it present in *Leucospermum* while I could find none in this genus. *Protea* is the only genus examined by the present author which is rich in arbutin and other compounds related to this hydroquinone glucoside.

All taxa of *Leucadendron* examined in this study gave a positive test for leucodrin and many gave a positive reaction for leudrin (the dihydroxy form of leucodrin described by Highet, Perold and Sokoloski, 1976). The general occurrence of leucodrin throughout this genus could relate it to species found in the last 4 sections of *Leucospermum* as they also contain this unusual phenolic dilactone. Based on flavonoid evidence there is a great deal of similarity between the two genera. Both contain only flavonols which suggests that from a phylogenetic point of view they are relatively primitive plants. Also, another similarity is the 3-0-point of attachment of the sugars which is the only glycoside attachment pattern found in both genera.

Another property in common is that both genera contain chiefly quercetin and isorhamnetin as aglycones with a very few species of each containing myricetin. It has been suggested that the presence of vicinal trihydroxy groups such as that found in myricetin is a primitive character in plant phylogeny (Harborne, 1967). Since more species of *Leucospermum* (5 out of 29 examined) contained myricetin than *Leucadendron* (3 species out of 59 examined) it could be suggested that *Leucospermum* is on the whole the more primitive of the two genera. This agrees with the findings of Elsworth and Martin and the distribution pattern of leucodrin reinforces this idea. It also supports the possibility that *Leucadendron* as a genus could have arisen from ancestors found in the more advanced sections of the genus *Leucospermum* (Rourke). However, this does not agree with the work of Johnson and Briggs (1975) who give *Leucadendron* and *Leucospermum* different ancestral types.

While comparing the flavonoid pattern of the two genera it should be pointed out that all *Leucospermum* spp. examined contained the rutinosides of quercetin and isorhamnetin while the rutinosides of these two aglycones occurred only sporadically in *Leucadendron*. Also, the arabinosides were common in *Leucospermum* but appear to be extremely rare in *Leucadendron*. The total flavonoid pattern of these two genera showed that they have many properties in common and are probably closely related but there are sufficient differences to separate them.

Leucoanthocyanins have been reported from all members of *Leucadendron* examined by Van Oudtshoorn and Elsworth and Martin. Because of this and also since it has been well documented that many members of the Proteaceae have been used as a source of tannin, the presence or absence of leucoanthocyanins has not been reported here. In view of the large amounts of anthocyanidins that are released on acid hydrolysis, it is probable that the tannins found in *Leucadendron* spp. are of the condensed type.

ACKNOWLEDGEMENTS

Professor G. W. Perold (Department of Chemistry, University of the Witwatersrand) is thanked for his encouragement and for his support in this work. The Director, Botanical Research Institute, Pretoria and Dr J. P. Rourke, National Botanic Gardens, Kirstenbosch are thanked for providing plant material.

REFERENCES

ELSWORTH, J. F. and MARTIN, K. R., 1971. Flavonoids of the Proteaceae: 1. A chemical contribution to studies on the evolutionary relationships in the S. African Proteoideae. *Jl S. Afr. Bot.* **37:** 199–212.

GLENNIE, C. W., 1979. Flavonoid glycosides of *Leucospermum* R. Br. *Jl S. Afr. Bot.* **45:** 185–191.

GLENNIE, C. W. and DAVIDSON, L. E., 1978. Flavonoids of the summer rainfall proteas and their chemotaxonomic significance. *Jl S. Afr. Bot.* **44:** 381–386.

HARBORNE, J. B., 1967. *Comparative Biochemistry of the Flavonoids.* London and New York: Academic Press.

———, 1975. The biochemical systematics of flavonoids. In: Harborne, J. B., Mabry, T. J. and Mabry, Helga. *The Flavonoids.* London: Chapman and Hall.

HEGNAUER, R., 1969. *Chemotaxonomie der Pflanzen.* Basel and Stuttgart: Birkhauser. Band 5, p. 407.

HIGHET, R. J., PEROLD, G. W. and SOKOLOSKI, E. A., 1976. Characterization of spiro-bislactonic phenolic metabolites of Proteaceae by ^{13}C nuclear magnetic resonance. *J. org. Chem.* **41:** 3860–3862.

JOHNSON, L. A. S. and BRIGGS, B. G., 1975. On the Proteaceae—the evolution and classification of a southern family. *J. Linn. Soc. Bot.* **70:** 83–182.

MURRAY, A. W. and BRADSHAW, R. W., 1966. Leucoglycodrin, a glycoside from *Leucadendron adscendens. Tetrahedron Lett.* **31:** 3773–3777.

Perold, G. W., Hodgkinson, A. J., Howard, A. S. and Kruger, P. E. J., 1972. Metabolites of Proteaceae. Part VI. The stereochemistry of reflexin and conocarpin. *J. chem. Soc. Perkin* **I:** 2457–2460.

Perold, G. W. and Pachler, K. G. R., 1966. The structure and chemistry of leucodrin. *J. chem. Soc.* (C): 1918–1923.

Plouvier, V., 1964. On the research of polyalcohols and cyanogenic glycosides of some members of Proteaceae. *Compt. Rend.* **259:** 665–668.

Rourke, J. P., personal communication.

Van Rheede van Oudtshorn, M. C. B., 1963. Distribution of phenolic compounds in some South African Proteaceae. *Planta med.* **11:** 399–406.

Williams, Ion J. M., 1972. A revision of the genus *Leucadendron* (Proteaceae). *Contr. Bol. Herb.* **3**.